doi:10.1038/nature17180

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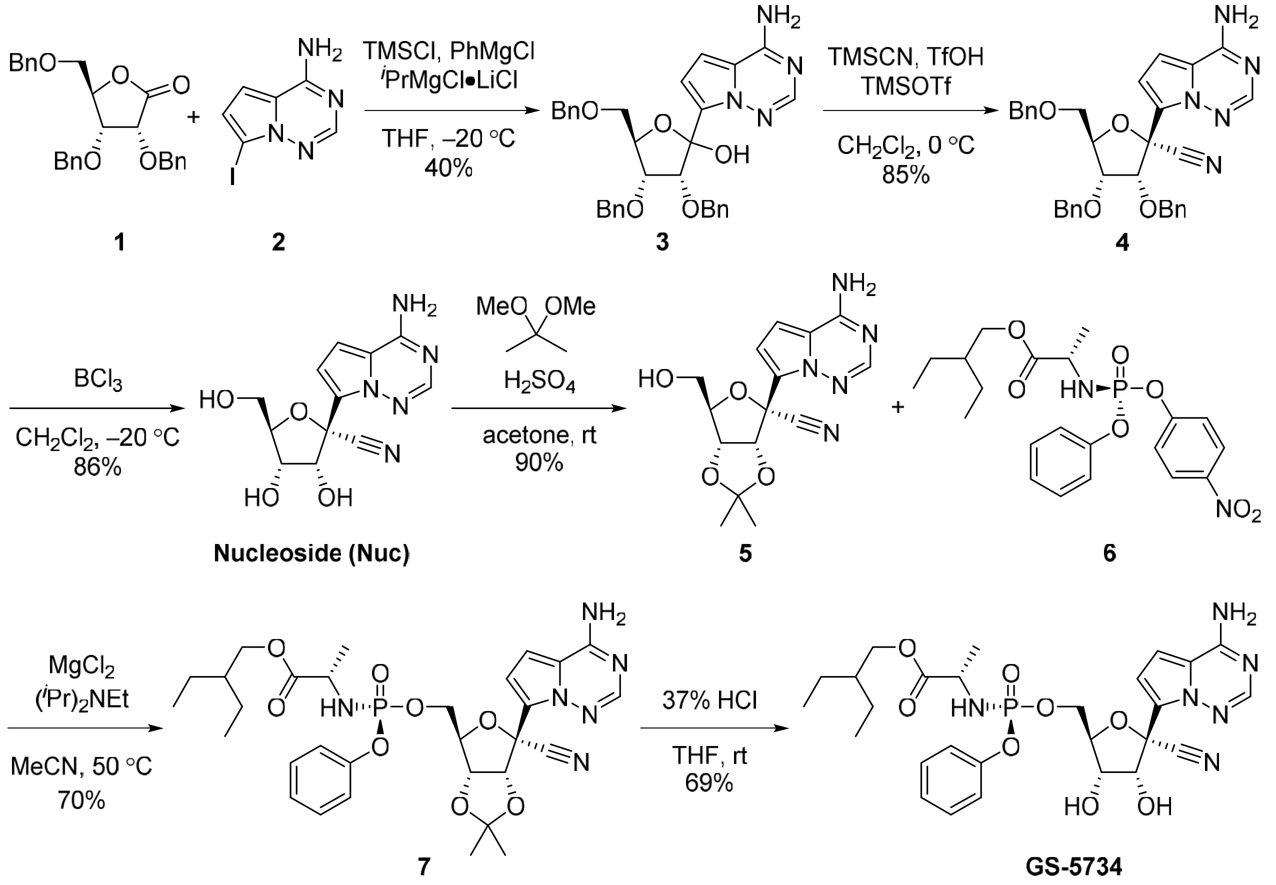
I. General Information

All organic compounds were synthesized at Gilead Sciences, Inc (Foster City, CA). unless otherwise noted. Commercially available solvents and reagents were used as received without further purification. 2,3,5-Tri-*O*-benzyl-D-ribono-1,4-lactone (**1**) was purchased from Carbosynth (Berkshire, UK). 7-iodopyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**2**) was prepared as previously described by Clarke, M. O. *et al*[38]. (*S*)-2-ethylbutyl 2-aminopropanoate hydrochloride (**8**) was prepared as previously described by Meppen, M. *et al*[39]. The radiolabeled analogue [^{14}C]GS-5734 (specific activity 58.0 mCi/mmol) was obtained from Moravek Biochemicals (Brea, CA) and was prepared in a similar manner described for GS-5734 using [^{14}C]TMSCN. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury Plus 400 MHz at room temperature, with tetramethylsilane as an internal standard. Proton nuclear magnetic resonance spectra are reported in parts per million (ppm) on the δ scale and are referenced from the residual protium in the NMR solvent (chloroform-d_1: δ 7.26, methanol-d_4: δ 3.31, water-d_2: δ 4.79, DMSO-d_6: δ 2.50). Data is reported as follows: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sep = septet, m = multiplet, br = broad, app = apparent), coupling constants (*J*) in Hertz, integration. Carbon-13 nuclear magnetic resonance spectra are reported in parts per million on the δ scale and are referenced from the carbon resonances of the solvent (chloroform-d_1: δ 77.16, methanol-d_4: δ 49.15, DMSO-d_6: δ 39.52). Data is reported as follows: chemical shift. No special nomenclature is used for equivalent carbons. Phosphorus-31 nuclear magnetic resonance spectra are reported in parts per million on the δ scale. Data is reported as follows: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet), coupling constants (*J*) in Hertz. Analytical thin–layer chromatography was performed using Merck KGaA Silica gel 60 F_{254} glassplates with UV visualization. Preparative normal phase silica gel chromatography was carried out using a Teledyne ISCO CombiFlash Companion instrument with silica gel cartridges. Purities of the final compounds were determined by high-performance liquid chromatography (HPLC) and were greater than 95% unless otherwise noted. HPLC conditions to assess purity were as follows: Agilent 1100 Series HPLC, Phenominex Gemini 5 µm C18 110Å, 50 × 4.6 mm column; 2-98% gradient of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile; flow rate, 2 mL/min; acquisition time, 6 min; wavelength, UV 214 and 254 nm. Analytical ion-exchange HPLC of the nucleoside triphosphate (**NTP**) was carried out as follows: Agilent 1100 Series HPLC, Thermo Scientific CNAPac™ PA-100 BioLC™ 4 × 250 mm column; 0-100% gradient of 0.5 M triethylammonium bicarbonate buffer in water; flow rate, 1 mL/min; acquisition time, 8 min; wavelength, UV 214 and 254 nm. High-resolution mass spectrometry (HRMS) was performed on an Agilent model 6230 Accurate Mass Time of Flight Mass Spectrometer featuring Agilent Jet Stream Thermal Focusing Technology, with an Agilent 1200 Rapid Resolution HPLC. HRMS chromatography was performed using an Agilent Zorbax Eclipse Plus C18 RRHD 1.8 µm, 2.1 × 50 mm column at 30 °C, with a 10-90% gradient of 0.05% trifluoroacetic acid in water and 0.05% trifluoroacetic acid in acetonitrile. **NTP** HRMS chromatography was performed using an Agilent Poroshell 120 PFP, 3.0, 50 mm, 2.7 µm LC column. Data processing was performed via Agilent MassHunter B.07 Qualitative Analysis. The reference masses used during the run were 118.086255 and 922.009798.

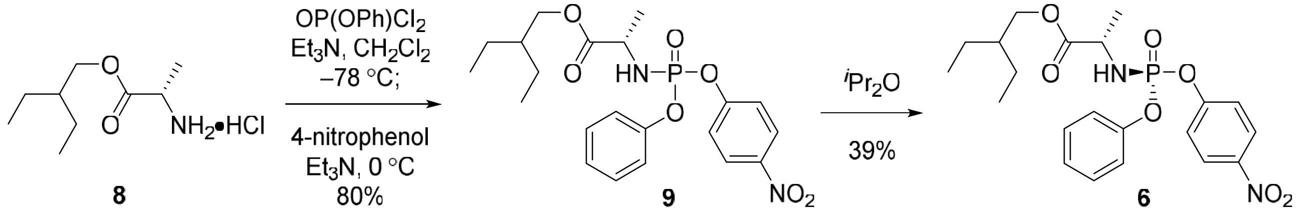
II. Preparation of Small Molecule Compounds

Nuc and **GS-5734** were prepared according to Scheme S1. Alternative methods for the synthesis of **Nuc** and GS-5734 mixture of phosphorus diastereoisomers have been described previously by Mackman, R. L. *et al*[40] and Metobo, S. E. *et al*[41]. (*S*)-2-ethylbutyl 2-(((*S*)-(4-nitrophenoxy)(phenoxy)phosphoryl)-amino)propanoate (**6**) was prepared according to Scheme S2. The nucleoside triphosphate (**NTP**) was prepared according to Scheme S3.

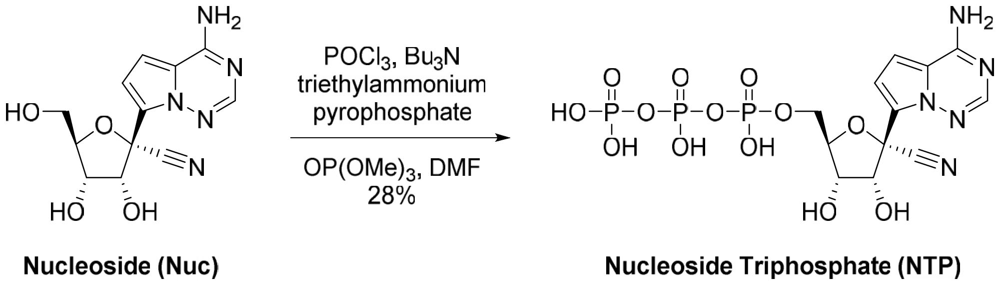
Scheme S1. Preparation of **Nuc** and **GS-5734**.



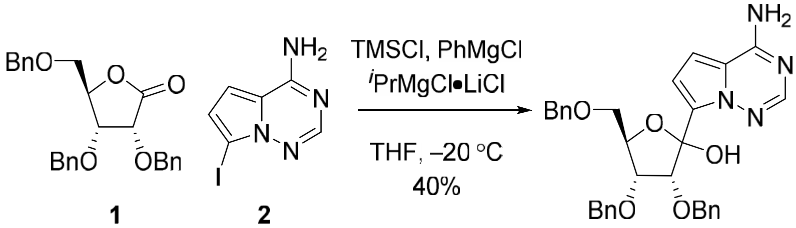
Scheme S2. Preparation of (*S*)-2-ethylbutyl 2-(((*S*)-(4-nitrophenoxy)(phenoxy)phosphoryl)-amino)propanoate (**6**).



Scheme S3. Preparation of nucleoside triphosphate (**NTP**).



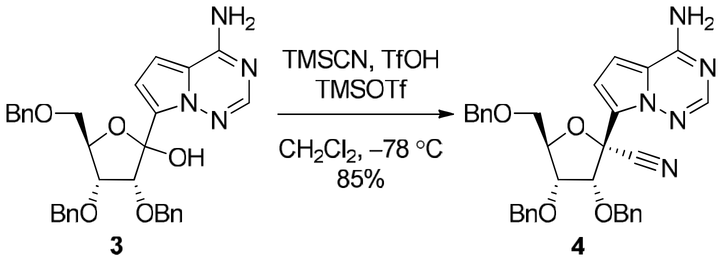
III. Experimental Procedures and Product Characterization



1 2 3

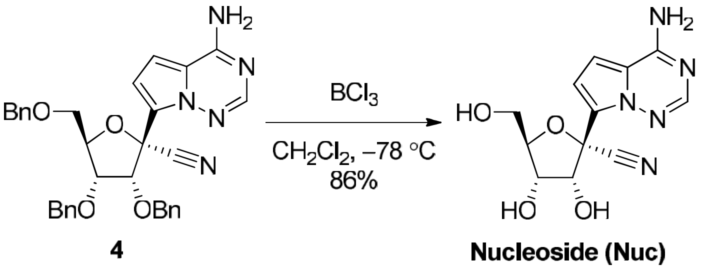
(3*R*,4*R*,5*R*)-2-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (3):

A solution of 7-iodopyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**2**, 6.21 g, 23.9 mmol, 1 equiv) was suspended in tetrahydrofuran (150 mL) under an argon atmosphere. TMSCl (6.07 mL, 23.9 mmol, 2.00 equiv) was added and the resulting mixture was stirred for 10 min at room temperature. The solution was cooled to approximately 0 °C, and PhMgCl (2 M in tetrahydrofuran, 23.9 mL, 47.8 mmol, 2.00 equiv) was added slowly. The reaction mixture was stirred for approximately 20 min, and ^{i}PrMgCl (1 M in tetrahydrofuran, 25.1 mL, 25.1 mmol, 1.00 equiv) was then added while maintaining an internal reaction temperature below 5 °C. After 15 min, the reaction mixture was cooled to approximately –20 °C and a solution of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (**1**, 10.0 g, 23.9 mmol, 1.00 equiv) in tetrahydrofuran (30 mL) was added slowly while maintaining an internal reaction temperature of approximately –20 °C. After 1 h, the reaction mixture was allowed to warm to 0 °C, and then quenched with methanol (20 mL) followed by acetic acid (20 mL) and water (20 mL). The resulting mixture was allowed to warm to room temperature and was then concentrated under reduced pressure. The resulting concentrate was partitioned between ethyl acetate (250 mL) and aqueous hydrochloric acid solution (1 M, 250 mL). The organic layer was separated and then washed with 10% aqueous sodium bicarbonate solution (250 mL) and brine (250 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude residue was subjected to silica gel chromatography eluting with 0-10% methanol in ethyl acetate to afford the mixture of isomers **3** (13.2 g, 41.5%) as an off-white solid. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.06 (br s, 2H), 7.99 (s, 1H), 7.37 – 7.22 (m, 11H), 7.19 – 7.10 (m, 3H), 7.03 – 6.97 (m, 2H), 6.95 (d, J = 4.8 Hz, 1H), 5.39 (d, J = 5.9 Hz, 1H), 5.05 (d, J = 5.2 Hz, 1H), 4.61 – 4.54 (m, 2H), 4.52 – 4.42 (m, 4H), 4.06 – 3.98 (m, 1H), 3.93 (dd, J = 5.9, 4.4 Hz, 1H), 3.69 (dd, J = 10.1, 3.4 Hz, 1H), 3.47 (dd, J = 10.0, 6.4 Hz, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 187.98, 155.88, 148.96, 138.63, 138.43, 138.14, 128.67, 128.14, 128.12, 127.82, 127.54, 127.44, 127.26, 127.21, 127.09, 118.60, 117.51, 103.15, 102.30, 81.91, 80.92, 72.50, 72.33, 71.74, 71.44, 69.42; HRMS (m/z): $[M]^+$ calcd for $C_{32}H_{32}N_4O_5$, 552.2373; found, 552.2382; HPLC: t_R = 3.293 min.



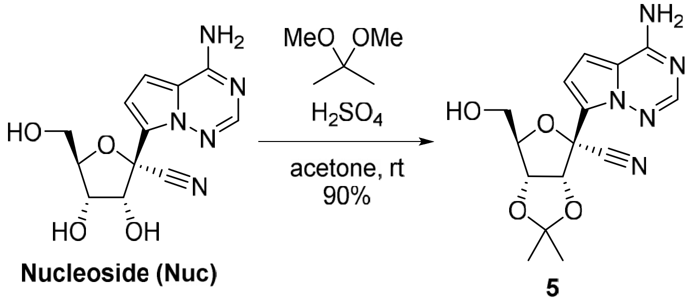
(2*R*,3*R*,4*R*,5*R*)-2-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-carbonitrile (4):

To a solution of (3*R*,4*R*,5*R*)-2-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (**3**, 57.9 g, 105 mmol, 1 equiv) in dichloromethane (100 mL) pre-cooled to –78 °C was added trifluoromethanesulfonic acid (18.3 mL, 206 mmol, 2.00 equiv). After the reaction was stirred for 10 min, TMSOTf (38.9 mL, 216 mmol, 2.10 equiv) was slowly added and the resulting mixture was stirred for 30 min at –78 °C. TMSCN (56.5 mL, 451 mmol, 4.00 equiv) was then added slowly and the mixture was stirred for 2 h. Triethylamine (50 mL) was added and the reaction mixture was allowed to warm to room temperature. Solid sodium bicarbonate (80 g) was then added followed by the slow addition of water (300 mL) and the resulting mixture was stirred for 10 min. The layers were then separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was subjected to silica gel chromatography eluting with 40-100% ethyl acetate in hexanes to afford the product **4** (58.9 g, 85%) as an off-white solid. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.99 – 7.82 (m, 3H), 7.37 – 7.23 (m, 15H), 6.88 (d, J = 4.5 Hz, 1H), 6.76 (d, J = 4.5 Hz, 1H), 4.91 (d, J = 5.0 Hz, 1H), 4.85 (d, J = 11.7 Hz, 1H), 4.77 (d, J = 11.7 Hz, 1H), 4.60 – 4.45 (m, 4H), 4.40 (q, J = 4.6 Hz, 1H), 4.12 (t, J = 5.4 Hz, 1H), 3.69 (dd, J = 11.1, 3.7 Hz, 1H), 3.59 (dd, J = 11.1, 4.7 Hz, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 155.54, 147.86, 138.08, 137.94, 137.32, 128.17, 128.14, 128.11, 127.93, 127.72, 127.52, 127.40, 122.63, 116.78, 116.73, 110.48, 100.81, 81.90, 79.25, 77.61, 76.26, 72.30, 72.27, 71.45, 68.79; HRMS (*m*/*z*): $[M]^+$ calcd for $C_{33}H_{31}N_5O_4$, 561.2376; found, 561.2394; HPLC: t_R = 3.581 min.



(2*R*,3*R*,4*S*,5*R*)-2-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-carbonitrile (Nuc):

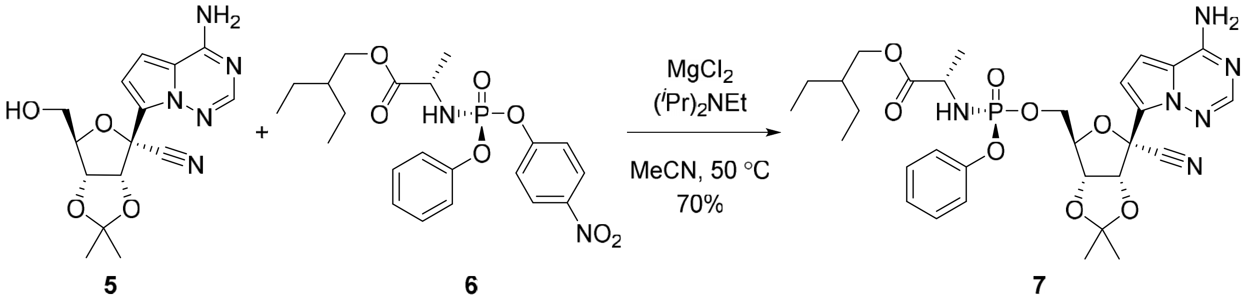
Boron trichloride (1 M, 35.0 mL, 35.0 mmol, 3.80 equiv) was slowly added to a solution of (2*R*,3*R*,4*R*,5*R*)-2-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-carbonitrile (**4**, 5.11 g, 9.10 mmol, 1 equiv) in anhydrous dichloromethane (50 mL) at –78 °C under an argon atmosphere. The reaction mixture was allowed to warm to –40 °C and was stirred for 2 h. The reaction mixture was cooled to –78 °C and methanol (10 mL) was added dropwise. A solution of triethylamine (13 mL) in methanol (20 mL) was added dropwise and the reaction mixture was allowed to warm to room temperature. The resulting mixture was concentrated under reduced pressure. The solid residue was slurried with hexanes (50 mL) and the supernatant was then decanted (3×). The remaining solid residue was suspended into methanol (50 mL) and was heated to 45 °C. Water (50 mL) was added, and the resulting mixture was concentrated at 45 °C under reduced pressure to remove the volatiles to a final volume of approximately 35 mL water. The mixture was allowed to cool to room temperature and the fine white solids were collected by vacuum filtration and dried in an oven at 70 °C overnight to afford the product **Nuc** (2.27 g, 86%). ^{1}H-NMR (400 MHz, water-*d*$_2$): *δ* 8.10 (s, 1H), 7.37 (d, *J* = 5.1 Hz, 1H), 7.14 (d, *J* = 4.8 Hz, 1H), 4.94 (d, *J* = 5.4 Hz, 1H), 4.42 (app q, *J* = 4.2 Hz, 1H), 4.35 (t, *J* = 5.1 Hz, 1H), 3.86 (dd, *J* = 12.8, 3.2 Hz, 1H), 3.79 (dd, *J* = 12.8, 4.7 Hz, 1H); ^{13}C-NMR (100 MHz, DMSO-*d*$_6$): *δ* 155.62, 147.87, 123.87, 117.34, 116.52, 110.77, 100.79, 85.42, 78.56, 74.24, 70.07, 60.94; HRMS (*m*/*z*): [M]$^+$ calcd for $C_{12}H_{13}N_5O_4$, 291.0968; found, 291.0967; HPLC: t_R = 0.350 min.



5

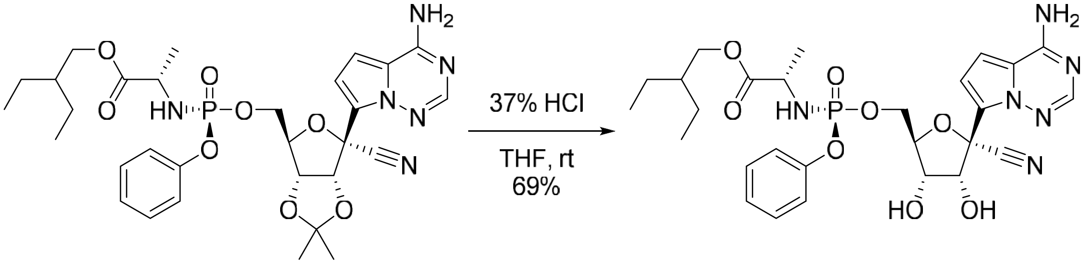
(3a*R*,4*R*,6*R*,6a*R*)-4-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonitrile (5):

Sulfuric acid (18 M, 1.4 mL, 26 mmol, 1.3 equiv) was added dropwise to a suspension of **Nuc** (5.8 g, 20 mmol, 1 equiv) and 2,2-dimethoxypropane (12 mL, 95 mmol, 4.8 equiv) in acetone (145 mL) at room temperature. The reaction mixture was stirred for 30 min, and was warmed to 45 °C. After 30 min, the reaction was allowed to cool to room temperature, and solid sodium bicarbonate (5.8 g) and water (5.8 mL) were sequentially added. The mixture was stirred for 15 min and then concentrated under reduced pressure. The residue was dissolved into ethyl acetate (150 mL) and water (50 mL). The organic layer was separated and the water layer was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried over anhydrous sodium sulfate and then concentrated under reduced pressure to afford product **5** (6.54 g, 99%), which was used directly in the next step without further purification. Analytically pure samples of **5** could be obtained through silica gel chromatography eluting with ethyl acetate. ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): δ 8.03 – 7.84 (m, 3H), 6.90 (app q, *J* = 4.6 Hz, 2H), 5.37 (d, *J* = 6.6 Hz, 1H), 5.01 (t, *J* = 5.7 Hz, 1H), 4.89 (dd, *J* = 6.6, 3.1 Hz, 1H), 4.31 (td, *J* = 5.2, 3.0 Hz, 1H), 3.59 – 3.45 (m, 2H), 1.63 (s, 3H), 1.37 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-*d*$_6$): δ 156.00, 148.58, 122.92, 117.37, 116.67, 115.83, 111.01, 101.28, 85.83, 84.33, 81.97, 80.37, 61.31, 26.30, 25.55; HRMS (*m*/*z*): $[M]^+$ calcd for $C_{15}H_{17}N_5O_4$, 331.1281; found, 331.1279; HPLC: t_R = 1.906 min.



(*S*)-2-ethylbutyl 2-(((*S*)-(((3a*R*,4*R*,6*R*,6a*R*)-6-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-6-cyano-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (7):

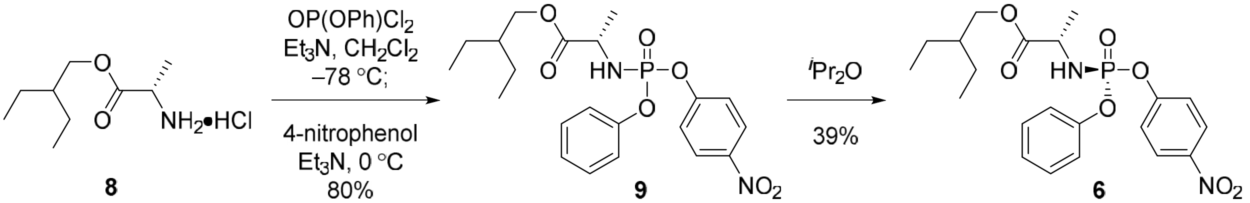
Acetonitrile (16 mL) was added to a mixture of (2*S*)-2-ethylbutyl 2-(((4-nitrophenoxy)(phenoxy)phosphoryl)amino)propanoate (**6**, 1.79 g, 3.98 mmol, 1.20 equiv), (3a*R*,4*R*,6*R*,6a*R*)-4-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carbonitrile (**5**, 1.10 g, 3.32 mmol, 1 equiv), and magnesium chloride (316 mg, 3.32 mmol, 1.00 equiv) at room temperature. The solution was heated to 50 °C for 10 min, and *N*,*N*-diisopropylethylamine (1.45 mL, 8.30 mmol, 2.50 equiv) was added. After 20 min, the reaction mixture was allowed to cool to room temperature, and then diluted with ethyl acetate (100 mL). The organic layer was washed with 5% aqueous citric acid solution (40 mL), saturated aqueous ammonium chloride solution (40 mL), 5% aqueous potassium carbonate solution (2 × 40 mL), and brine (40 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was subjected to silica gel chromatography eluting with 0-100% ethyl acetate in hexanes to afford the product **7** (1.5 g, 70%). ^{1}H-NMR (400 MHz, methanol-d_4): δ 7.86 (s, 1H), 7.30 – 7.23 (m, 2H), 7.17 – 7.10 (m, 3H), 6.89 (q, J = 4.6 Hz, 2H), 5.34 (d, J = 6.6 Hz, 1H), 4.99 (dd, J = 6.6, 3.5 Hz, 1H), 4.60 – 4.53 (m, 1H), 4.36 – 4.24 (m, 2H), 4.02 (dd, J = 10.9, 5.8 Hz, 1H), 3.92 (dd, J = 10.9, 5.7 Hz, 1H), 3.84 (dq, J = 9.7, 7.1 Hz, 1H), 1.70 (s, 3H), 1.50 – 1.42 (m, 1H), 1.40 (s, 3H), 1.36 – 1.23 (m, 7H), 0.86 (t, J = 7.4 Hz, 6H); ^{13}C-NMR (100 MHz, methanol-d_4): δ 174.92, 174.87, 157.21, 152.08, 152.01, 148.46, 130.66, 130.65, 126.08, 126.07, 124.56, 121.35, 121.30, 118.31, 117.78, 117.01, 112.34, 102.43, 85.68, 84.90, 84.82, 83.07, 82.57, 68.09, 66.96, 51.46, 41.69, 26.52, 25.55, 24.22, 24.20, 20.46, 20.39, 11.34, 11.31; ^{31}P-NMR (162 MHz, methanol-d_4): δ 3.38 (s); HRMS (m/z): [M]$^+$ calcd for $C_{30}H_{39}N_6O_8P$, 642.2567; found, 642.2584; HPLC: t_R = 3.349 min.



7 **GS-5734**

(*S*)-2-ethylbutyl 2-(((*S*)-(((2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (GS-5734):

To a stirred solution of (*S*)-2-ethylbutyl 2-(((*S*)-(((3a*R*,4*R*,6*R*,6a*R*)-6-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-6-cyano-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (**7**, 12.9 g, 20.0 mmol, 1 equiv) in tetrahydrofuran (100 mL) was added 37% aqueous hydrochloric acid solution (20 mL) slowly at 0 °C. The reaction mixture was allowed to warm to room temperature. After 5 h, the reaction mixture was diluted with water (100 mL) and adjusted to pH=8 by the addition of saturated aqueous sodium bicarbonate solution (200 mL). The resulting mixture was extracted with ethyl acetate (100 mL), and the organic extract was then washed with brine (50 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was subjected to silica gel chromatography eluting with 0-100% ethyl acetate in hexanes to afford **GS-5734** (8.3 g, 69%). ^{1}H-NMR (400 MHz, methanol-d_4): δ 7.86 (s, 1H), 7.33 – 7.26 (m, 2H), 7.21 – 7.12 (m, 3H), 6.91 (d, J = 4.6 Hz, 1H), 6.87 (d, J = 4.6 Hz, 1H), 4.79 (d, J = 5.4 Hz, 1H), 4.43 – 4.34 (m, 2H), 4.28 (ddd, J = 10.3, 5.9, 4.2 Hz, 1H), 4.17 (t, J = 5.6 Hz, 1H), 4.02 (dd, J = 10.9, 5.8 Hz, 1H), 3.96 – 3.85 (m, 2H), 1.49 – 1.41 (m, 1H), 1.35 – 1.27 (m, 8H), 0.85 (t, J = 7.4 Hz, 6H). ^{13}C-NMR (100 MHz, methanol-d_4): δ 174.98, 174.92, 157.18, 152.14, 152.07, 148.27, 130.68, 126.04, 125.51, 121.33, 121.28, 117.90, 117.58, 112.29, 102.60, 84.31, 84.22, 81.26, 75.63, 71.63, 68.10, 67.17, 67.12, 51.46, 41.65, 24.19, 20.56, 20.50, 11.33, 11.28.; ^{31}P-NMR (162 MHz, methanol-d_4): δ 3.66 (s); HRMS (m/z): [M]$^+$ calcd for $C_{27}H_{35}N_6O_8P$, 602.2254; found, 602.2274; HPLC: t_R = 2.911 min.

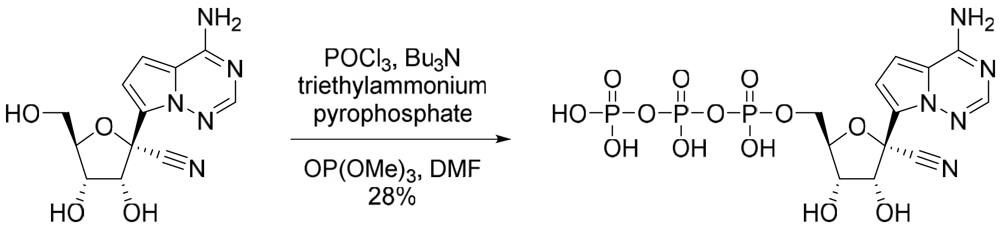


(*S*)-2-ethylbutyl 2-(((*S*)-(4-nitrophenoxy)(phenoxy)phosphoryl)amino)propanoate (6):

(*S*)-2-ethylbutyl 2-aminopropanoate hydrochloride (**8**, 26.0 g, 124 mmol, 1.10 equiv) was suspended in dichloromethane (200 mL) and the resulting mixture was cooled to –78 °C. Phenyl dichlorophosphate (18.5 mL, 124 mmol, 1.10 equiv) was then added followed by the slow addition of triethylamine (17.2 mL, 124 mmol, 1.10 equiv). The resulting mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was then cooled to 0 °C and 4-nitrophenol (15.5 g, 112 mmol, 1 equiv) was added followed by the slow addition of triethylamine (17.2 mL, 124 mmol, 1.10 equiv). The resulting mixture was allowed to warm to room temperature and stirred for 3 h. The reaction mixture was then concentrated under reduced pressure and the crude residue was subjected to silica gel chromatography eluting with 0-30% ethyl acetate in hexanes to afford **9** (33 g, 66%) as a colorless semi-solid. ^{1}H-NMR (400 MHz, chloroform-d_1): δ 8.22 (d, J = 9.0 Hz, 2H), 7.37 (dt, J = 13.7, 7.8 Hz, 4H), 7.28 – 7.16 (m, 3H), 4.21 – 4.10 (m, 1H), 4.10 – 3.99 (m, 2H), 3.95 – 3.85 (m, 1H), 1.55 – 1.45 (m, 1H), 1.45 – 1.14

(m, 7H), 0.87 (t, *J* = 7.4 Hz, 6H); ^{13}C-NMR (100 MHz, chloroform-d_1): δ 173.27, 173.24, 173.18, 173.16, 155.68, 155.63, 155.62, 155.57, 150.39, 150.33, 150.27, 129.97, 129.95, 129.95, 125.70, 125.67, 125.60, 120.94, 120.92, 120.89, 120.87, 120.23, 120.19, 120.18, 120.14, 67.89, 67.87, 50.63, 50.61, 40.33, 40.32, 23.26, 23.24, 21.17, 21.13, 21.08, 11.03, 11.01; ^{31}P-NMR (162 MHz, chloroform-d_1): δ –3.04 (s), –3.10 (s); HRMS (*m*/*z*): $[M]^+$ calcd for $C_{21}H_{27}N_2O_7P$, 450.1556; found, 450.1571; HPLC: t_R = 4.258 min.

(2*S*)-2-ethylbutyl 2-(((4-nitrophenoxy)(phenoxy)phosphoryl)amino)propanoate (**9**, 10.5 g, 23.3 mmol, 1 equiv) was suspended in diisopropyl ether (42 mL) and gently stirred. After 22 h, the white solids were collected by vacuum filtration and dried to afford product **6** (4.10 g, 39%) as a single diastereoisomer. The absolute stereochemistry of **6** was unambiguously assigned through X-ray crystallographic analysis (Figure S1). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.34 – 8.26 (m, 2H), 7.54 – 7.47 (m, 2H), 7.44 – 7.37 (m, 2H), 7.27 – 7.19 (m, 3H), 6.68 (dd, *J* = 13.6, 10.0 Hz, 1H), 4.07 – 3.94 (m, 1H), 3.92 (d, *J* = 5.7 Hz, 2H), 1.40 (app p, *J* = 6.2 Hz, 1H), 1.30 – 1.19 (m, 7H), 0.79 (t, *J* = 7.4 Hz, 6H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 172.78, 172.73, 155.58, 155.52, 150.18, 150.11, 144.06, 129.80, 125.74, 125.15, 121.06, 121.00, 120.19, 120.15, 66.19, 50.00, 39.65, 22.50, 22.48, 19.68, 19.61, 10.73, 10.71. ^{31}P-NMR (162 MHz, DMSO-d_6): δ –1.25 (s); HRMS (*m*/*z*): $[M]^+$ calcd for $C_{21}H_{27}N_2O_7P$, 450.1556; found, 450.1571; HPLC: t_R = 4.258 min.



((2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxytetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate (NTP):

To a solution of **Nuc** (1.00 g, 3.43 mmol, 1 equiv) in $OP(OMe)_3$ (15 mL) at 0 °C was added $POCl_3$ (827 mg, 5.39 mmol, 1.57 equiv). The reaction mixture was stirred at 0 °C for 4 h, and solution of pyrophosphate tributylamine salts (3.00 g, 5.47 mmol, 1.59 equiv) in acetonitrile (10 mL) was added followed by the addition of tributylamine (3.11 g, 16.8 mmol, 4.89 equiv). The reaction mixture was stirred at 0 °C for 30 min, and then quenched by the addition of triethylammonium bicarbonate buffer (1 M, 40 mL). The resulting mixture was stirred at room temperature for 30 min, and then triethylamine (4 mL) was added. The mixture was stirred an additional 30 min, and concentrated under reduced pressure and co-evaporated with water (2×). The residue was dissolved in water (40 mL) and the solution was subjected to ion-exchange column chromatography (Column: Thermo Scientific, DNAP PA-100, 4 × 250 mm; eluting with water, then 5-35% 1 M triethylammonium bicarbonate buffer in water). The product fractions were combined, concentrated under reduced pressure and co-evaporated with water. The residue was dissolved in water (40 mL) and was resubjected to ion-exchange column chromatography (Column: Thermo Scientific, DNAP PA-100, 4 × 250 mm; eluting with water, then 5-35% 1 M triethylammonium bicarbonate buffer in water). The product fractions were combined and concentrated under reduced pressure to afford the product **NTP** triethylamonium salt (770 mg, 24%, 1.8 equiv triethylamonium) as an off-white solid. ^{1}H-NMR (400 MHz, water-d_2, signals for triethylammonium denoted by *): δ 7.93 (br s, 1H), 7.11 – 7.03 (m, 1H), 6.95 – 6.84 (m, 1H), 5.07 – 4.98 (m, 1H), 4.65 – 4.57 (m, 1H), 4.57 – 4.50 (m, 1H), 4.31 – 4.15 (m, 1H), 4.13 – 4.00 (m, 1H), 3.16 – 3.00 (m, 6H*), 1.30 – 1.13 (m, 9H*); ^{13}C-NMR (100 MHz, water-d_2, signals for triethylammonium denoted by *): δ 155.49, 147.21, 122.98, 117.11, 116.45, 111.08, 102.22, 85.32, 76.16, 74.75, 70.14, 64.64, 46.36*, 8.32*; ^{31}P-NMR (162 MHz, D_2O): δ –5.56 (d, J = 19.4 Hz), –10.96 (d, J = 19.2 Hz), –21.45 (t, J = 19.4 Hz); HRMS (m/z): $[M]^+$ calcd for $C_{12}H_{16}N_5O_{13}P_3$, 530.9957; found, 530.9957; ion-exchange HPLC: t_R = 5.422 min.

IV. Single Crystal X-ray Diffraction

Single Crystal X-ray Diffraction of Compound 6

The single crystal X-ray diffraction studies were carried out on a Bruker APEX CCD diffractometer equipped with Mo K_α radiation (λ = 0.71078Å). Crystals of compound **6** suitable for X-ray crystallographic analysis were grown from slow evaporation of a diisopropyl ether solution to afford thin colorless needles, which were then heat-cycled in a Technobis Crystal-16 instrument to afford larger crystals. A 0.24 × 0.10 × 0.08 mm colorless block of compound **6** was mounted on a Cryoloop with Paratone® oil. Data were collected in a nitrogen gas stream at 100(2) K using ϕ and ϖ scans. The diffractometer was a Bruker Ultra D8 platform equipped with a rotating-anode source and microfocus optics. The crystal-to-detector distance was 50 mm, and exposure time was 5 s/frame using a scan width of 0.5°. Data collection was 96.2% complete to 28.3° in θ. A total of 6610 reflections were collected covering the indices, $-6 \leq h \leq 3$, $-13 \leq k \leq 21$, $-27 \leq l \leq 31$. 4213 reflections were found to be symmetry independent, with a R_{int} of 0.0444. Indexing and unit cell refinement indicated a primitive, orthorhombic lattice. The space group was found to be $P2_12_12_1$. The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program. Solution by direct methods (SHELXS) produced a complete phasing model consistent with the proposed structure. All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-13). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-13. The absolute stereochemistry of compound **6** is unambiguous. The X-ray crystallographic coordinates and structure factor files for compound **6** have been deposited in the Cambridge Structural Database (accession number 1445315, http://www.ccdc.cam.ac.uk/).

Figure S1. Thermal ellipsoid representation of compound **6**.

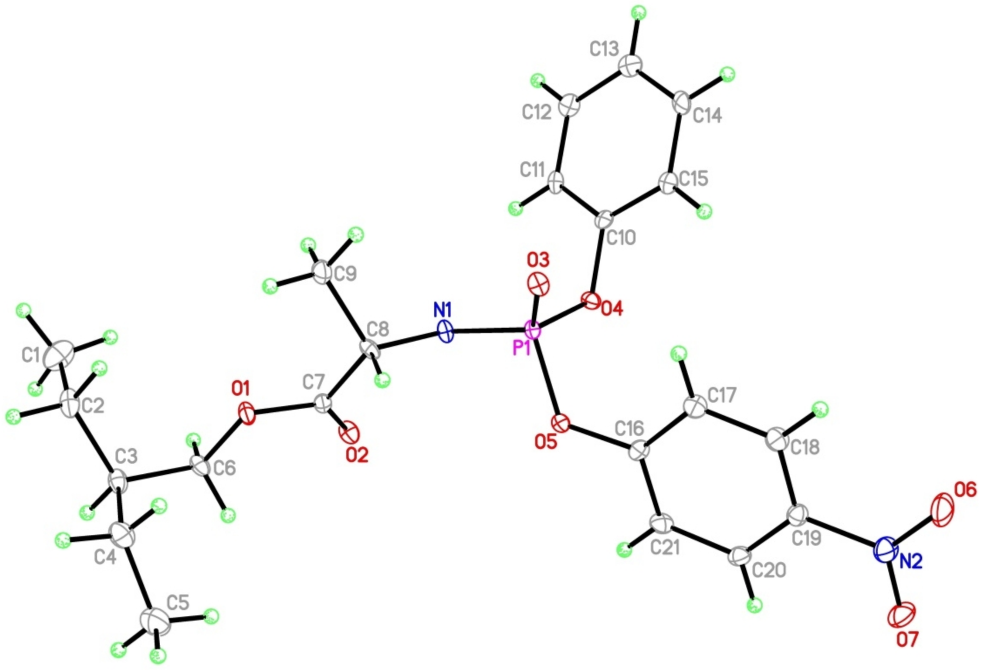
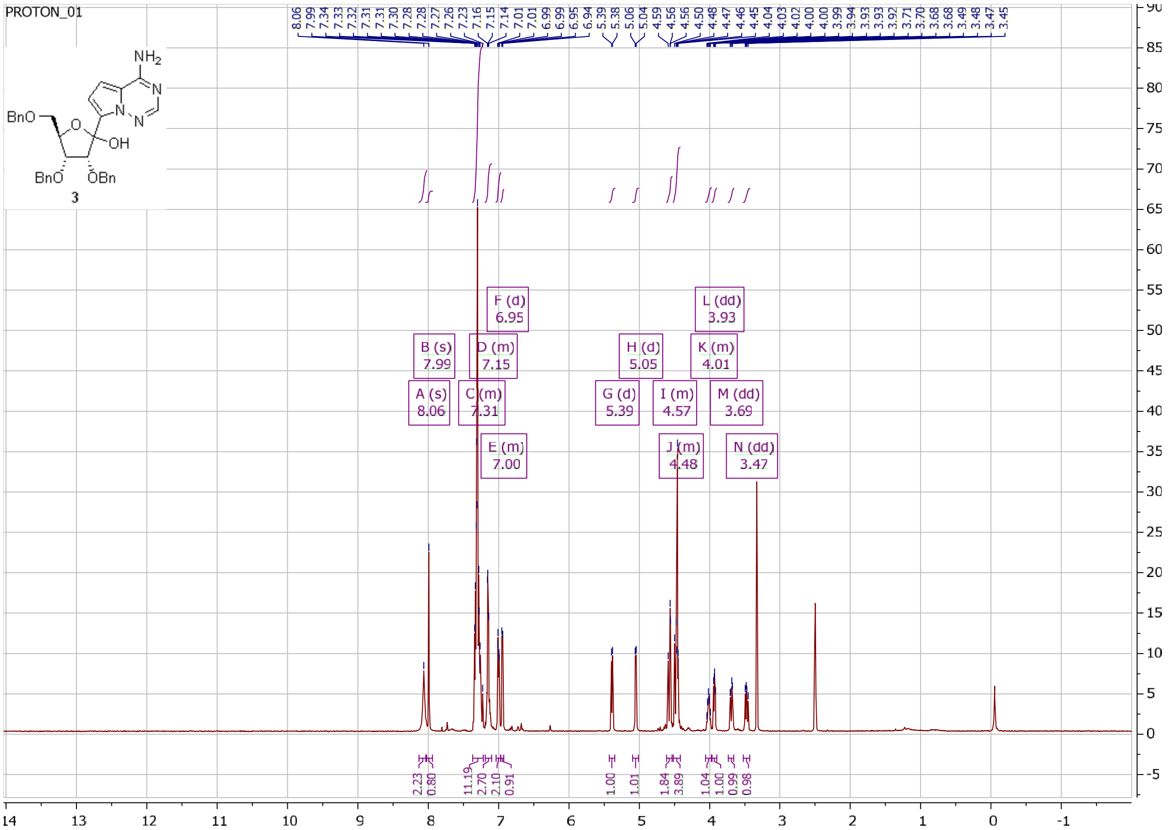


Table S1. Crystal data and structure refinement for compound **6**.

Empirical formula	$C_{21}H_{27}N_2O_7P$	
Formula weight	450.41	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 5.2493(4) Å	α = 90°
	b = 16.5797(12) Å	β= 90°
	c = 25.2623(17) Å	γ = 90°
Volume	2198.6(3) Å^3	
Z	4	
Density (calculated)	1.361 Mg/m^3	
Absorption coefficient	0.170 mm^{-1}	
F(000)	952	
Crystal size	0.24 × 0.10 × 0.08 mm^3	
Theta range for data collection	2.457 to 28.272°.	
Index ranges	$-6 \leq h \leq 3$, $-13 \leq k \leq 21$, $-27 \leq l \leq 31$	
Reflections collected	6610	
Independent reflections	4213 [R(int) = 0.0444]	
Completeness to theta = 28.3°	96.2 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4213 / 0 / 283	
Goodness-of-fit on F^2	1.019	
Final R indices [I>2sigma(I)]	R1 = 0.0488, wR2 = 0.1058	
R indices (all data)	R1 = 0.0622, wR2 = 0.1142	
Absolute structure parameter	-0.10(10)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.433 and -0.445 e.Å^{-3}	

V. NMR Spectra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N
BnO
O
OH
BnO
OBn
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7.99
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7.31
7.31
7.30
7.28
7.28
7.27
7.26
7.23
7.16
7.15
7.14
7.01
7.01
6.99
6.99
6.95
6.94
5.39
5.38
5.06
5.04
4.59
4.56
4.56
4.50
4.48
4.47
4.46
4.45
4.04
4.03
4.02
4.00
4.00
3.99
3.94
3.93
3.93
3.92
3.71
3.70
3.68
3.68
3.49
3.48
3.47
3.45
A (s) 8.06
B (s) 7.99
C (m) 7.31
D (m) 7.15
E (m) 7.00
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G (d) 5.39
H (d) 5.05
I (m) 4.57
J (m) 4.48
K (m) 4.01
L (dd) 3.93
M (dd) 3.69
N (dd) 3.47
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11.19
2.70
2.10
0.91
1.00
1.01
1.84
3.89
1.04
1.00
0.99
0.98
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
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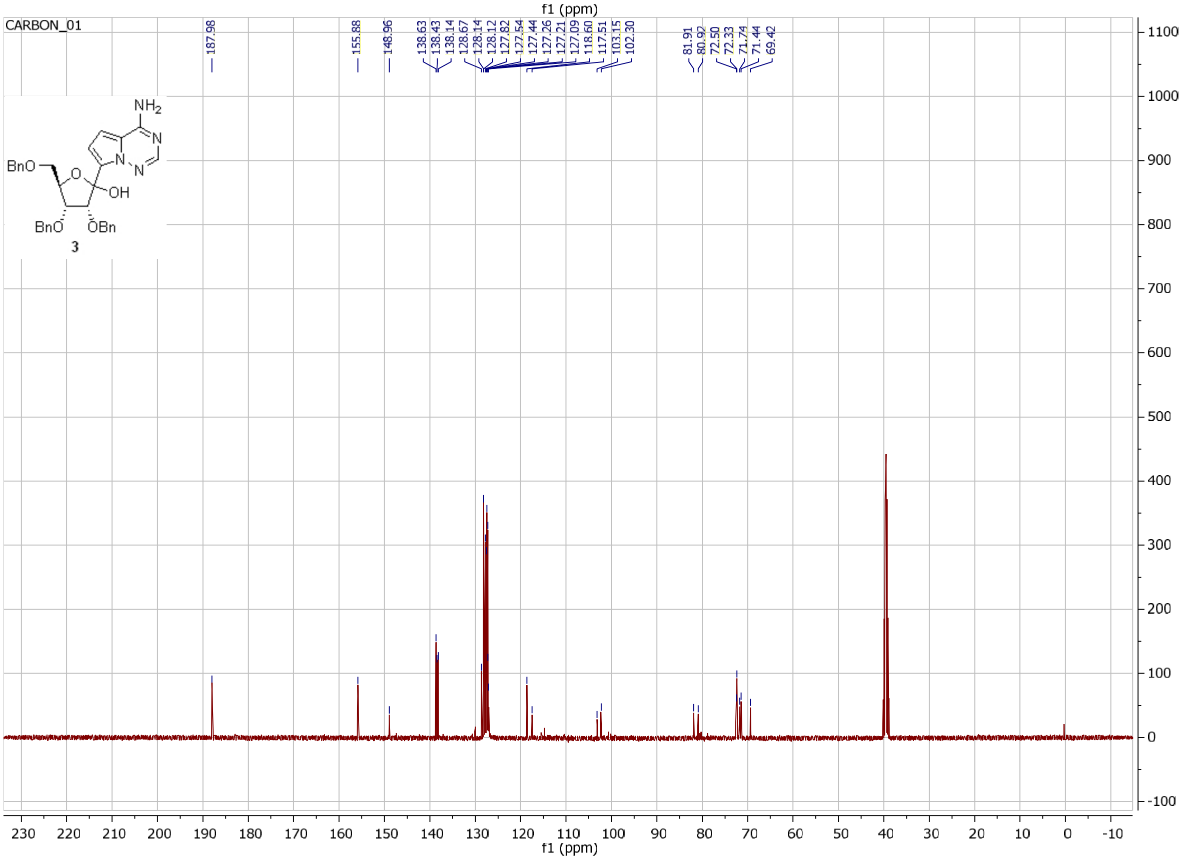
f1 (ppm)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127.09
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117.51
103.15
102.30
81.91
80.92
72.50
72.33
71.74
71.44
69.42
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
230
220
210
200
190
180
170
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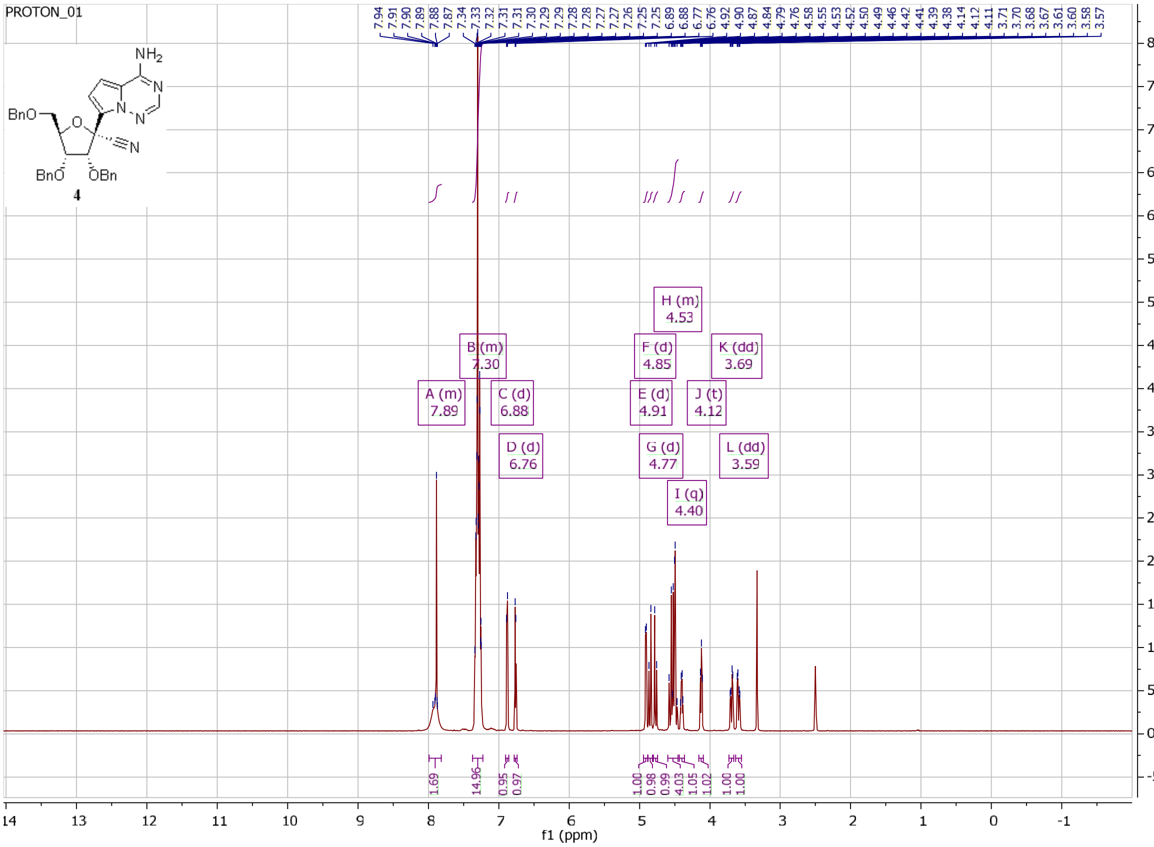
f1 (ppm)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O
N
BnO
OBn
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C (d) 6.88
D (d) 6.76
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F (d) 4.85
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I (q) 4.40
J (t) 4.12
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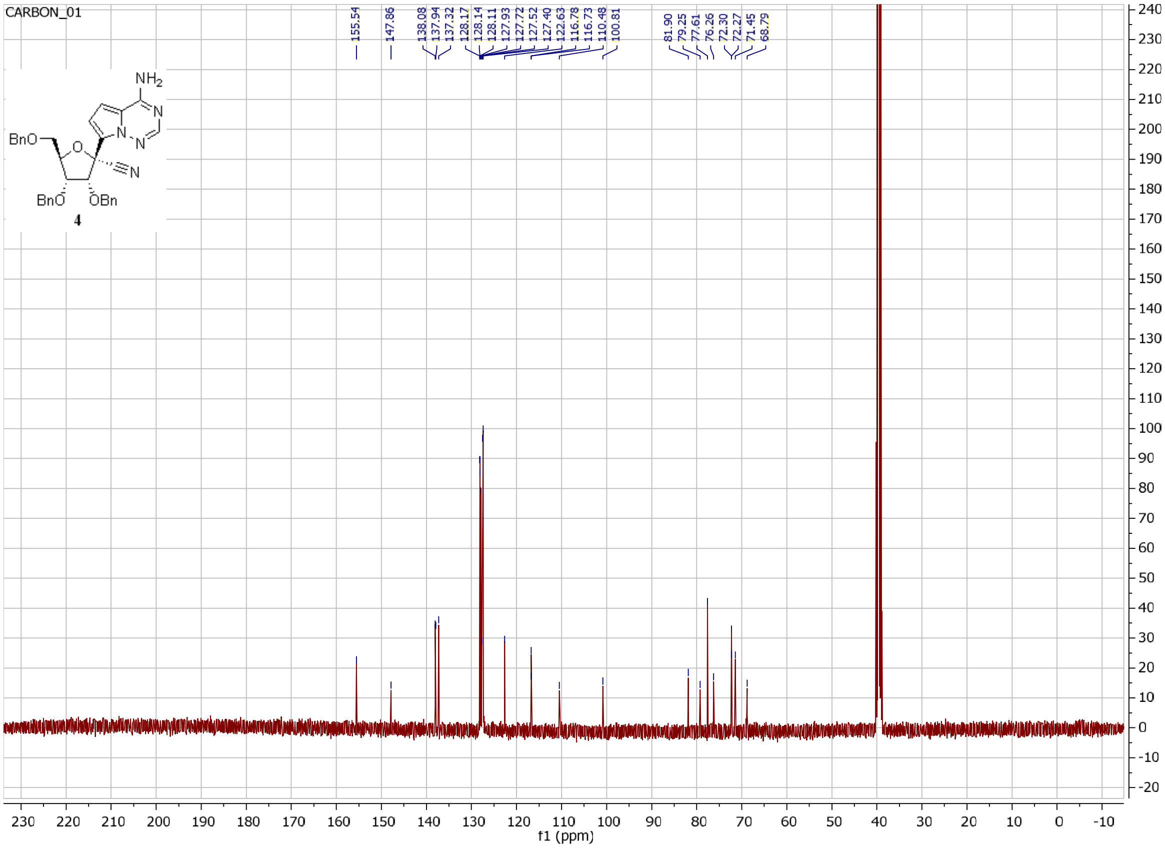
f1 (ppm)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BnO
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BnO
OBn
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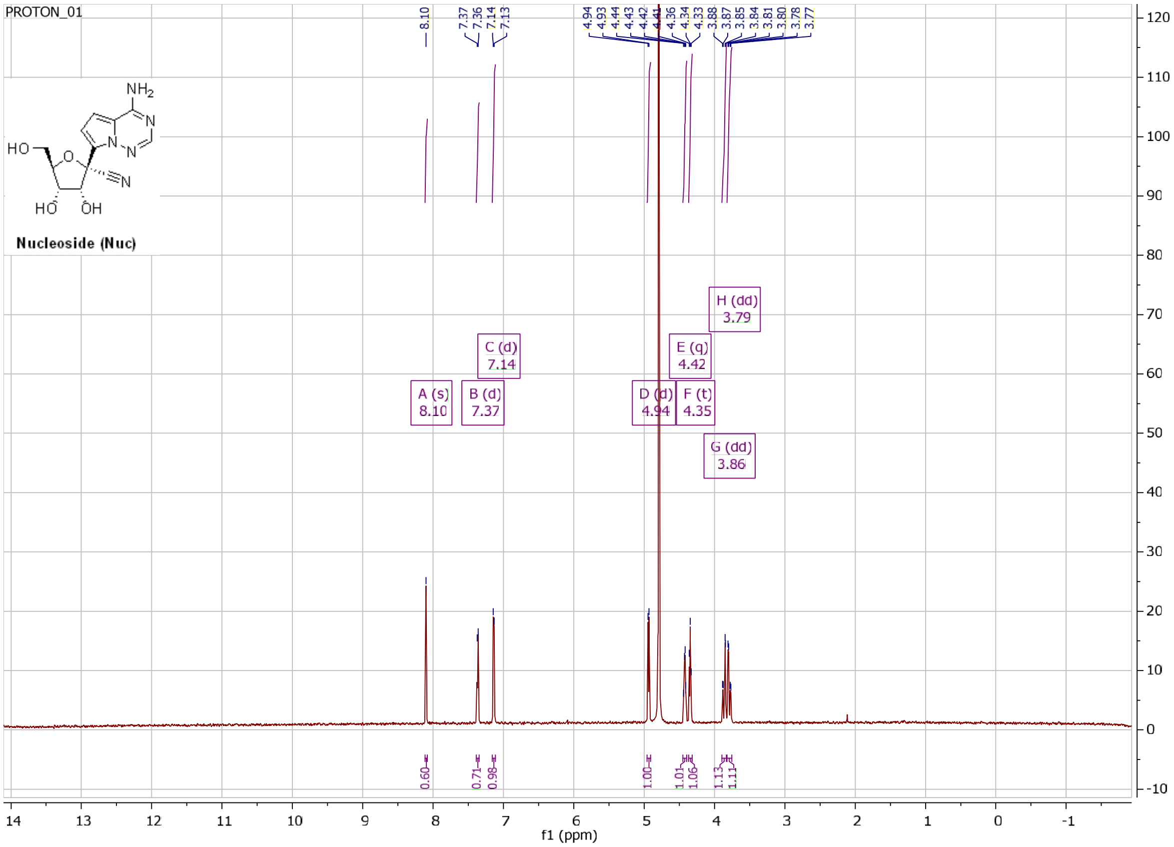
f1 (ppm)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N
HO
OH
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7.13
4.94
4.93
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4.43
4.42
4.41
4.36
4.34
4.33
3.88
3.87
3.85
3.84
3.81
3.80
3.78
3.77
A (s) 8.10
B (d) 7.37
C (d) 7.14
D (d) 4.94
E (q) 4.42
F (t) 4.35
G (dd) 3.86
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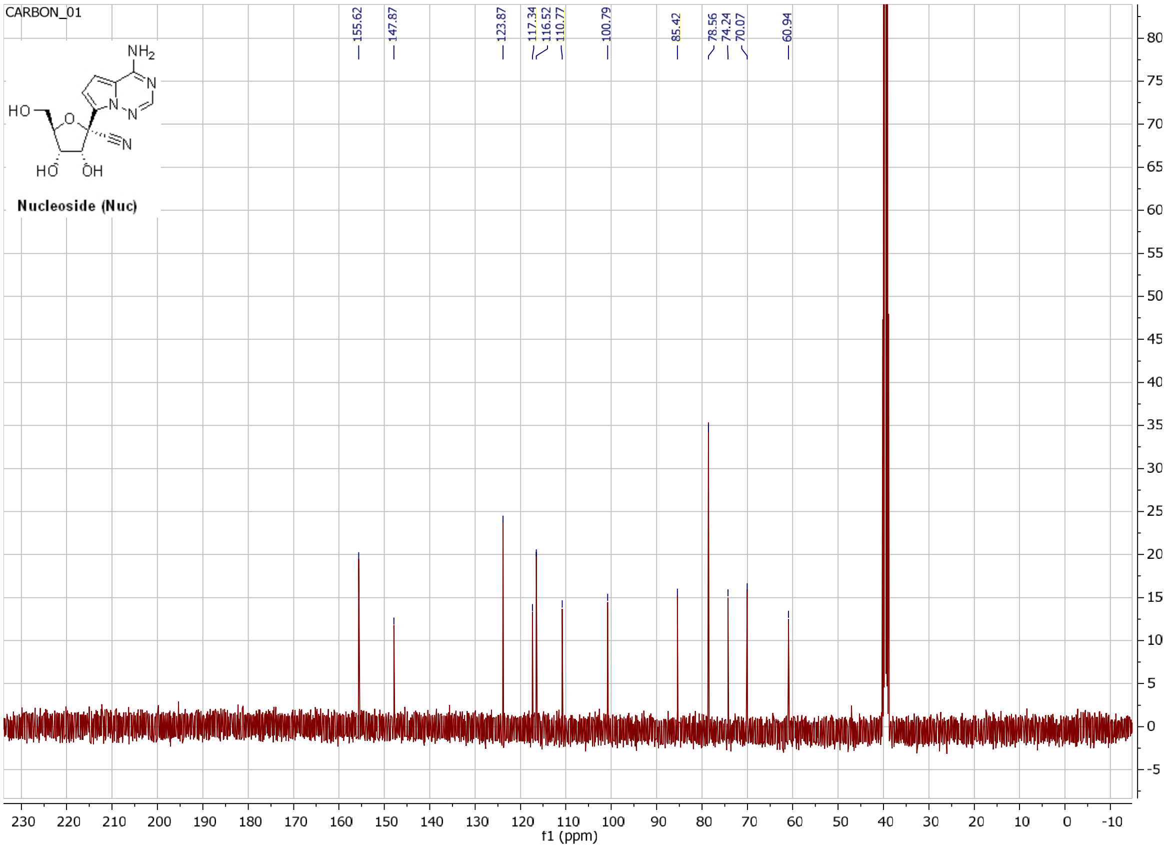
1.11
f1 (ppm)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HO
O
N
HO
OH
Nucleoside (Nuc)
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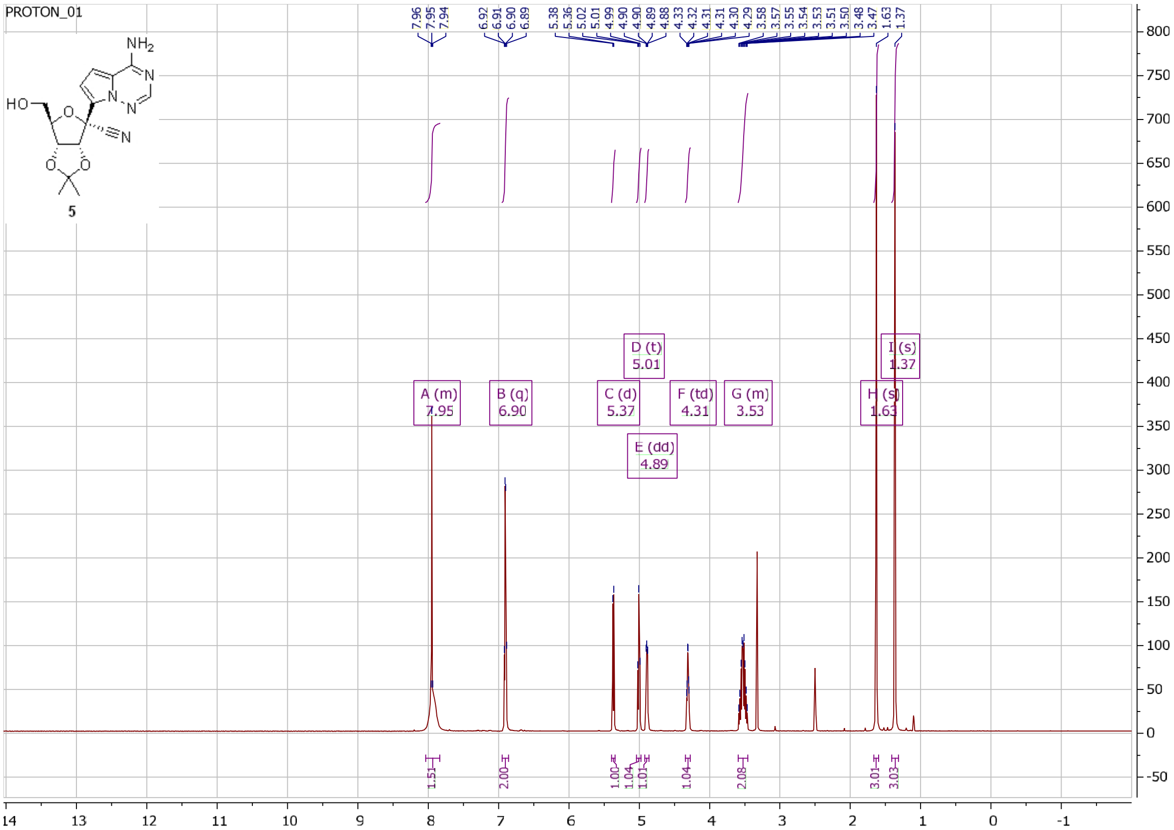
60.94
f1 (ppm)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6.89
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5.36
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4.89
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4.32
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4.30
4.29
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3.54
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3.51
3.50
3.48
3.47
1.63
1.37
A (m) 7.95
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C (d) 5.37
D (t) 5.01
E (dd) 4.89
F (td) 4.31
G (m) 3.53
H (s) 1.63
I (s) 1.37
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1.00
1.04
1.01
1.04
2.08
3.01
3.03
f1 (ppm)
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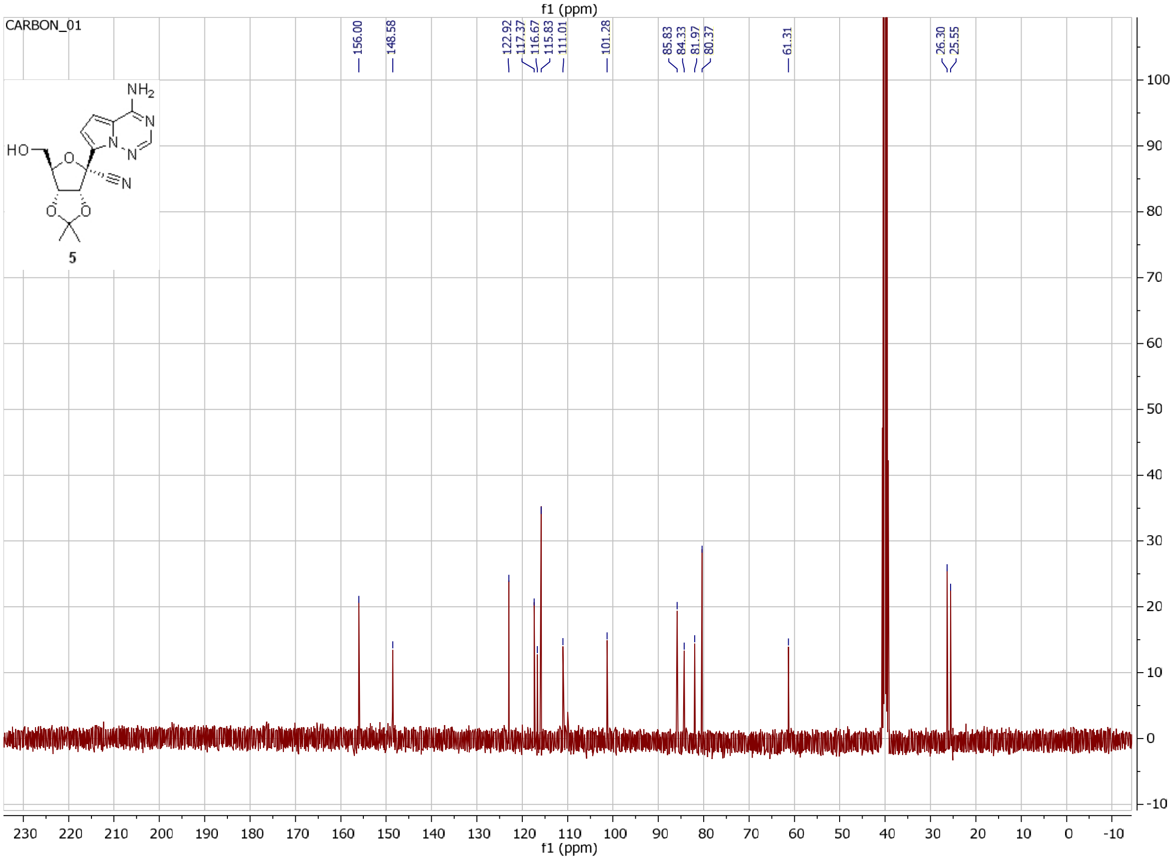
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111.01
101.28
85.83
84.33
81.97
80.37
61.31
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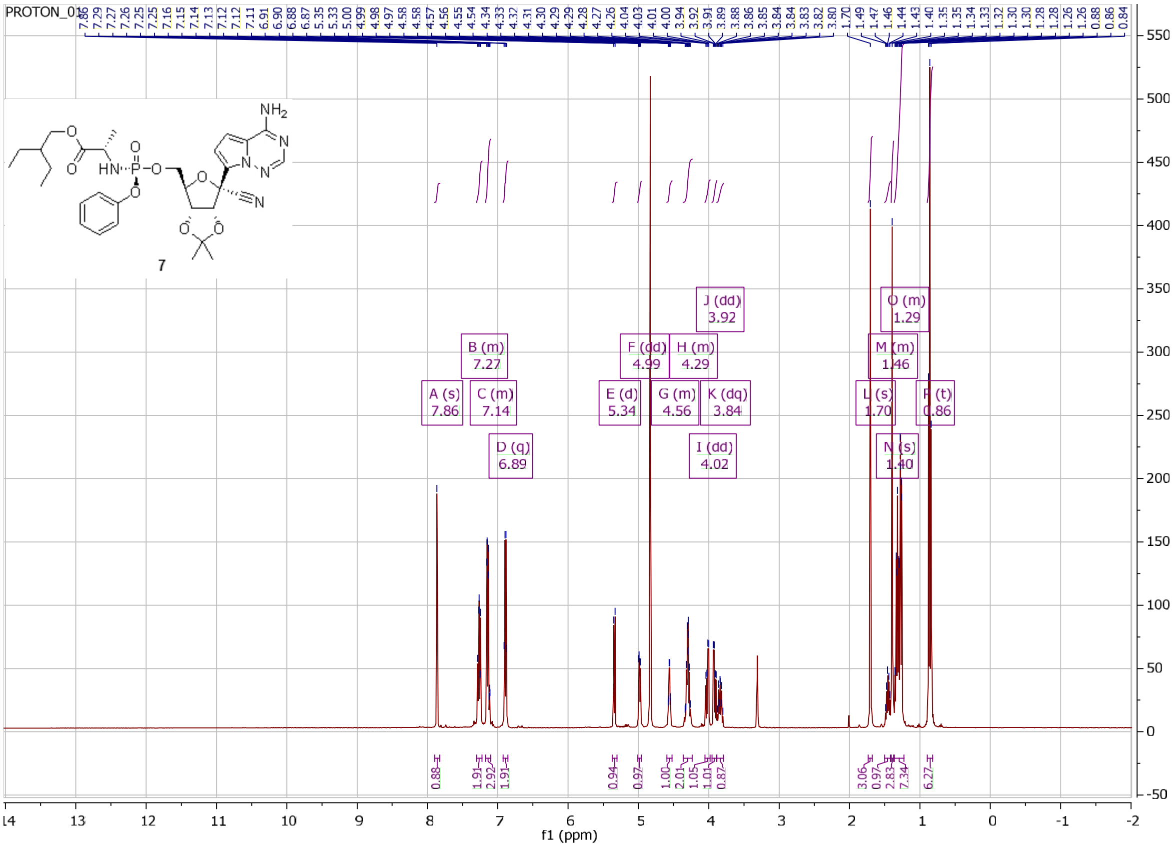
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NH2
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HN
P
O
N
N
N
N
7
A (s) 7.86
B (m) 7.27
C (m) 7.14
D (q) 6.89
E (d) 5.34
F (dd) 4.99
G (m) 4.56
H (m) 4.29
I (dd) 4.02
J (dd) 3.92
K (dq) 3.84
L (s) 1.70
M (m) 1.46
N (s) 1.40
O (m) 1.29
P (t) 0.86
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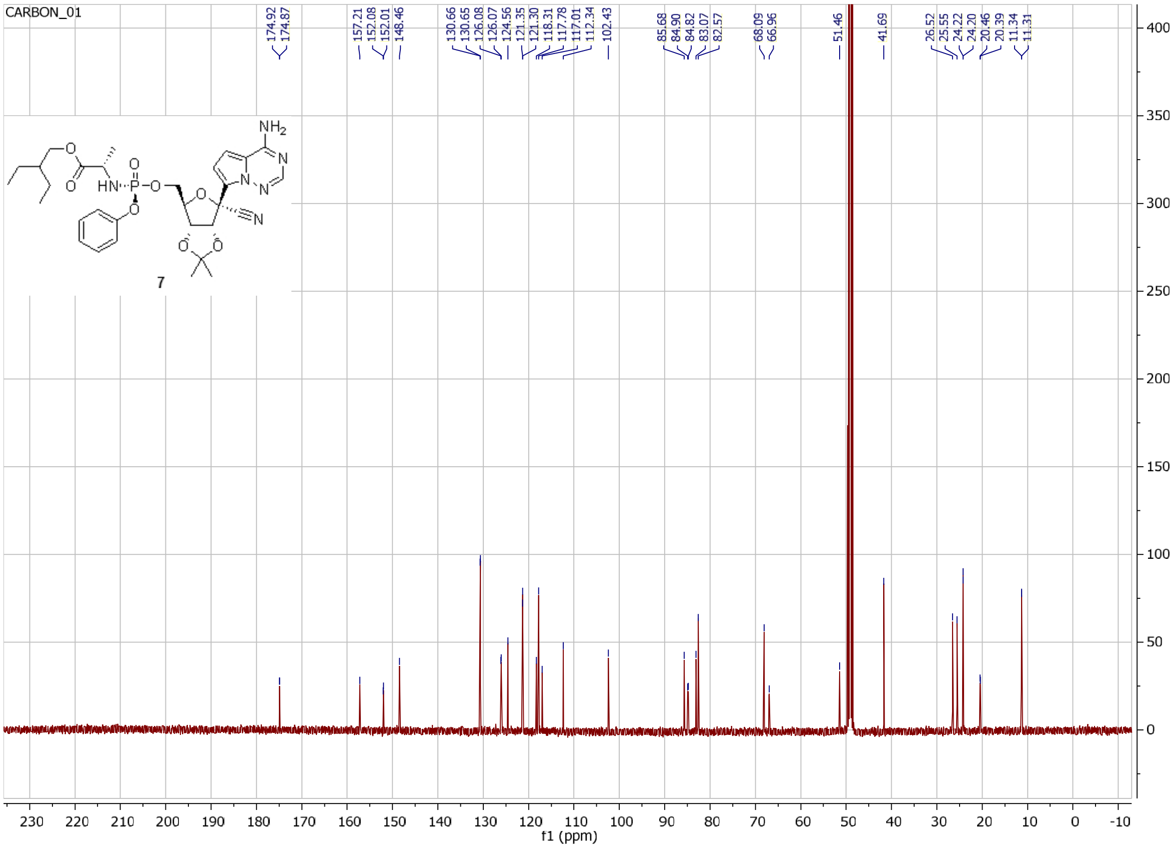
f1 (ppm)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NH2
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HN
P
O
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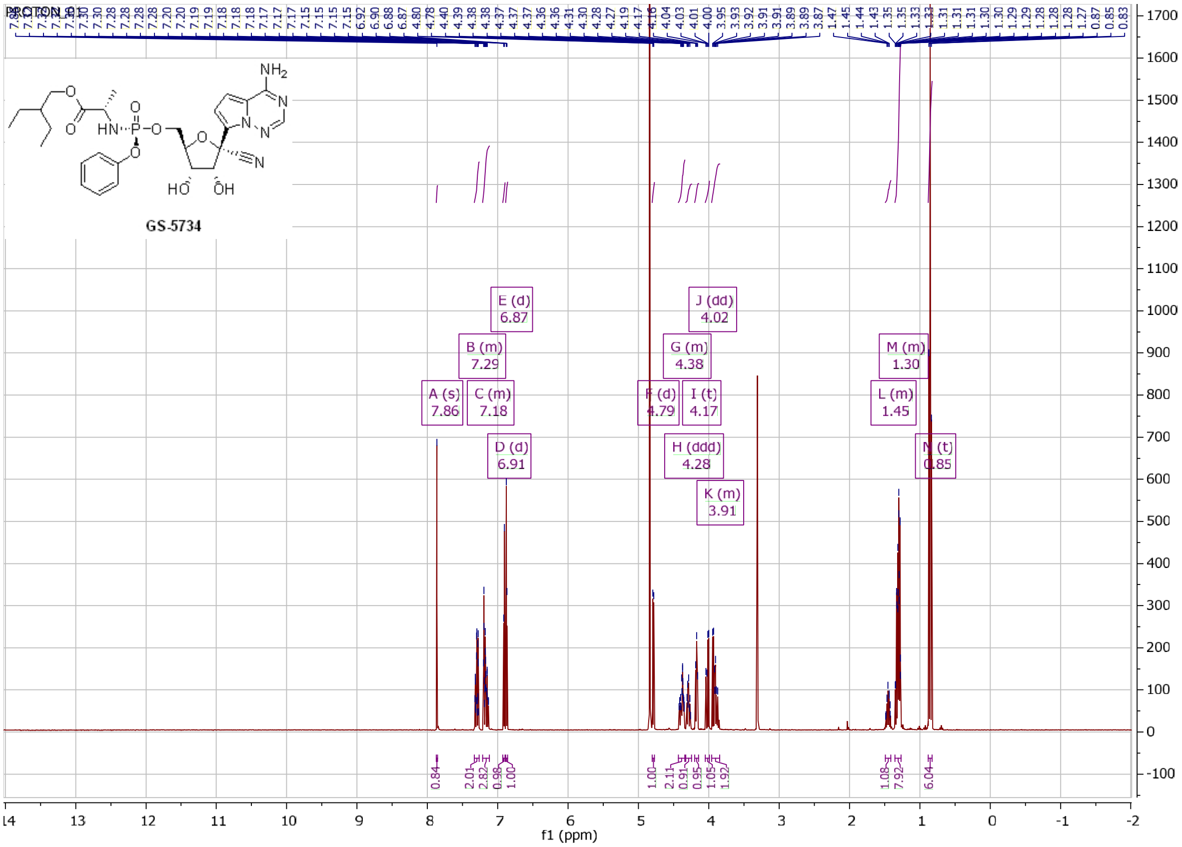
f1 (ppm)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4.27
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1.35
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1.33
1.33
1.31
1.31
1.31
1.30
1.30
1.29
1.29
1.28
1.28
1.28
1.27
0.87
0.85
0.83
NH2
N
N
N
O
O
O
HN
P
O
O
O
O
N
HO
OH
GS-5734
E (d) 6.87
J (dd) 4.02
B (m) 7.29
G (m) 4.38
M (m) 1.30
A (s) 7.86
C (m) 7.18
F (d) 4.79
I (t) 4.17
L (m) 1.45
D (d) 6.91
H (ddd) 4.28
N (t) 0.85
K (m) 3.91
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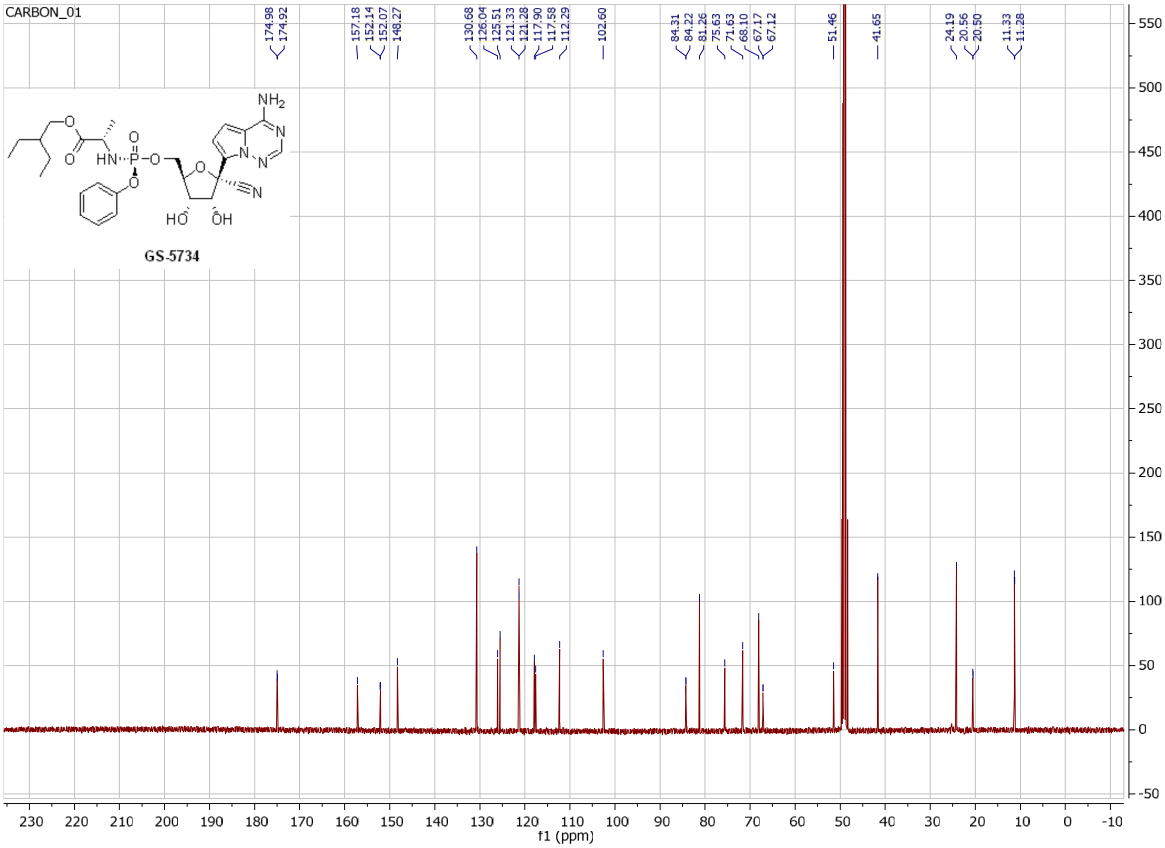
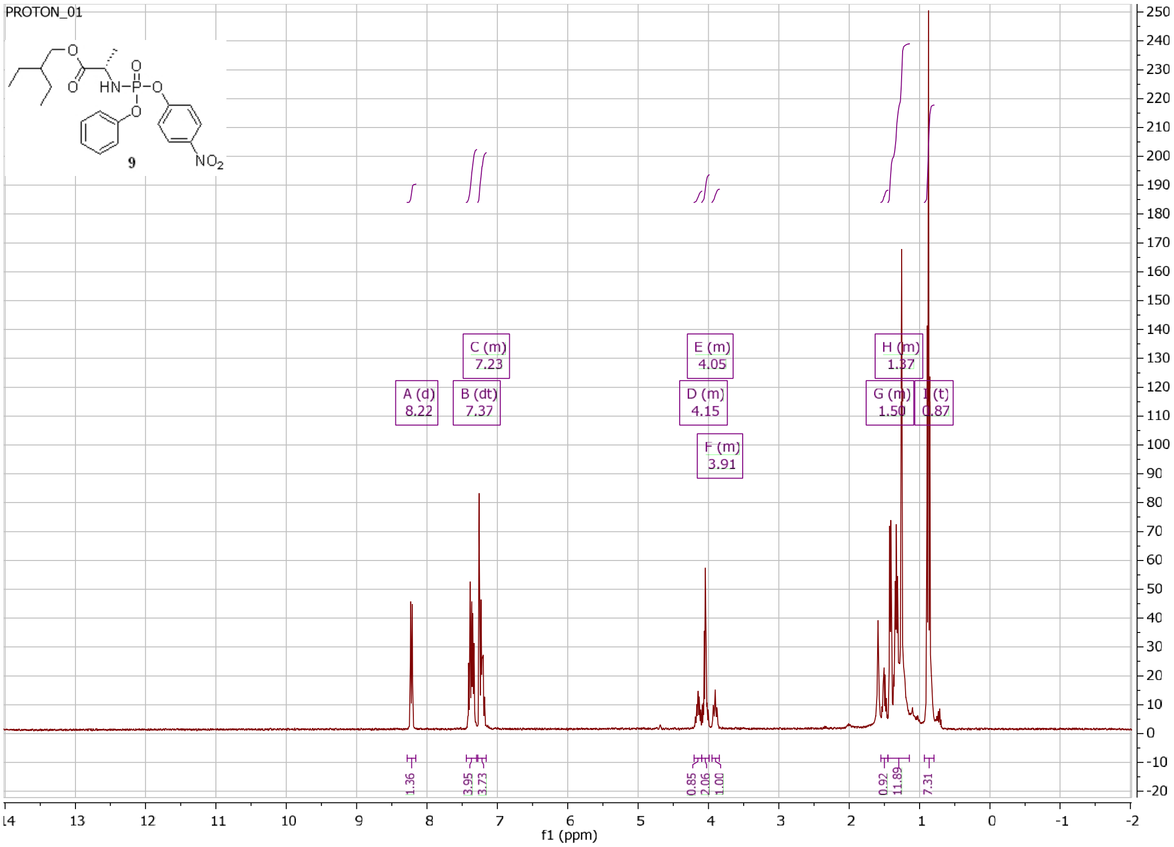
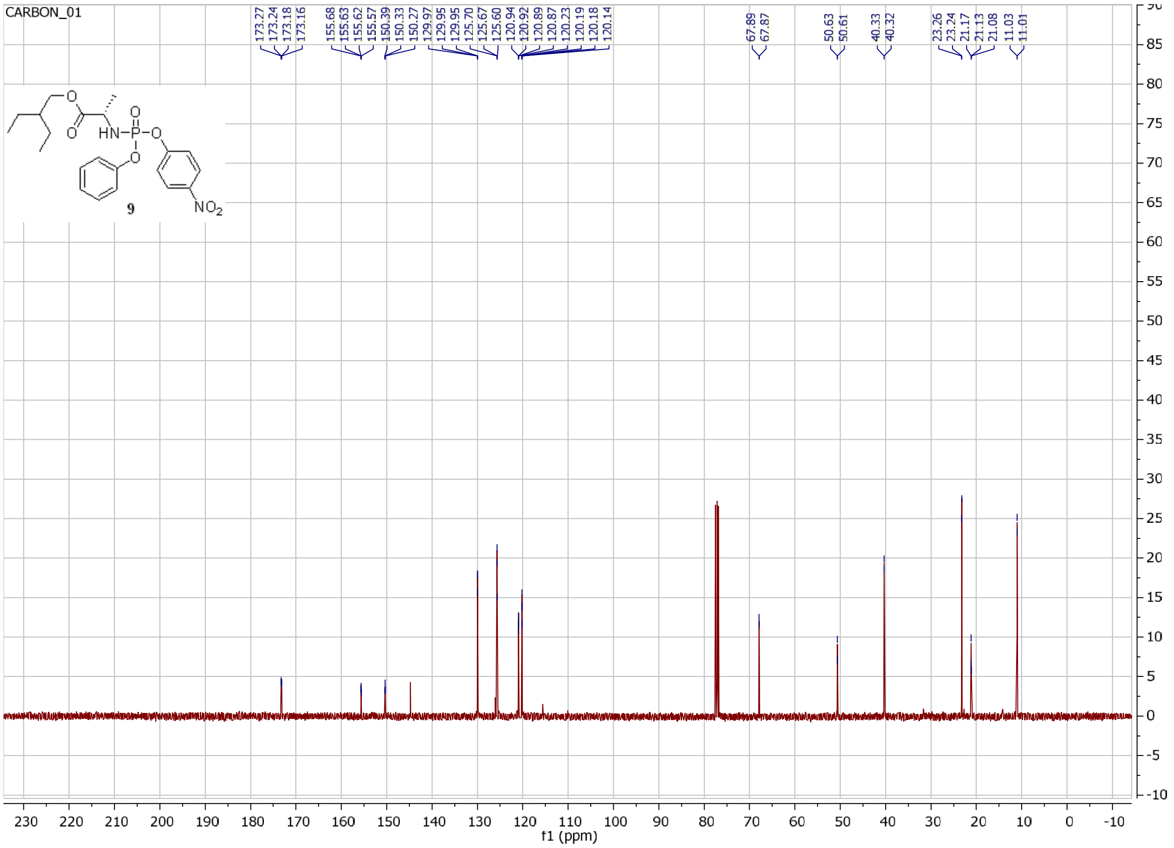
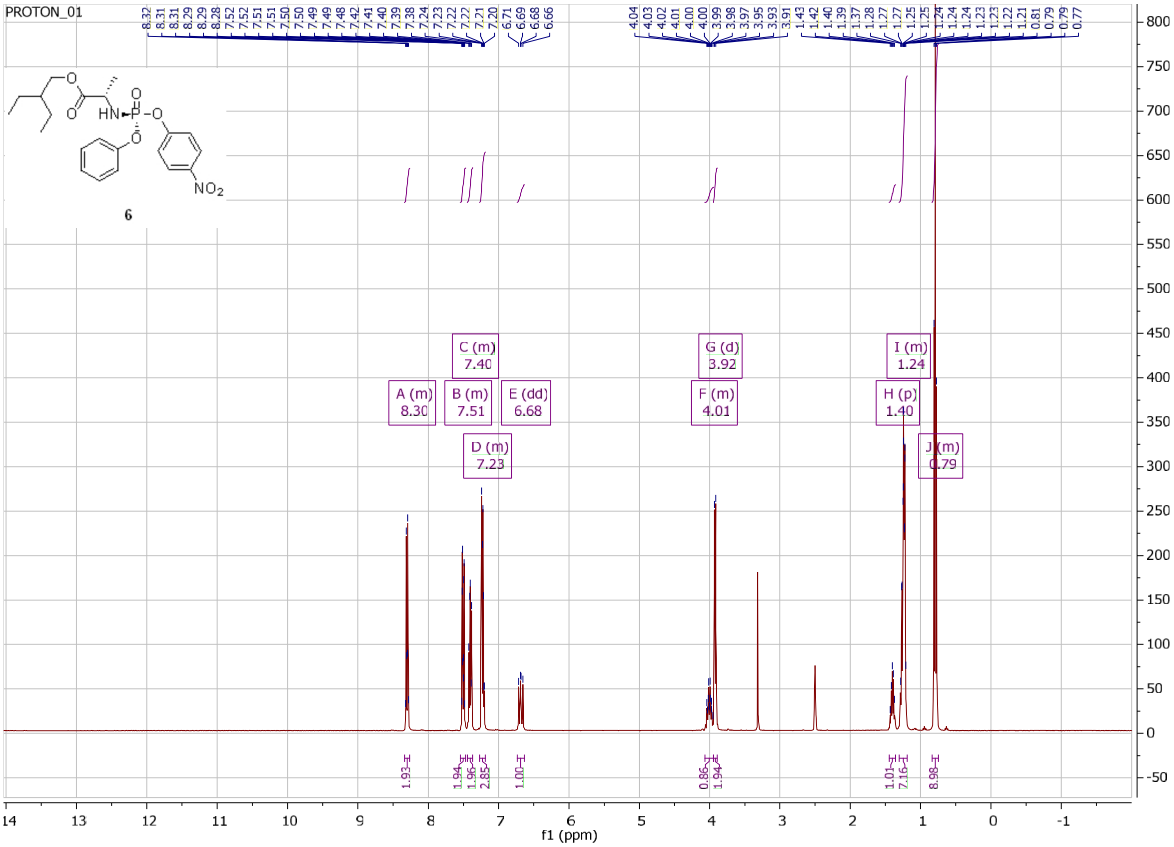
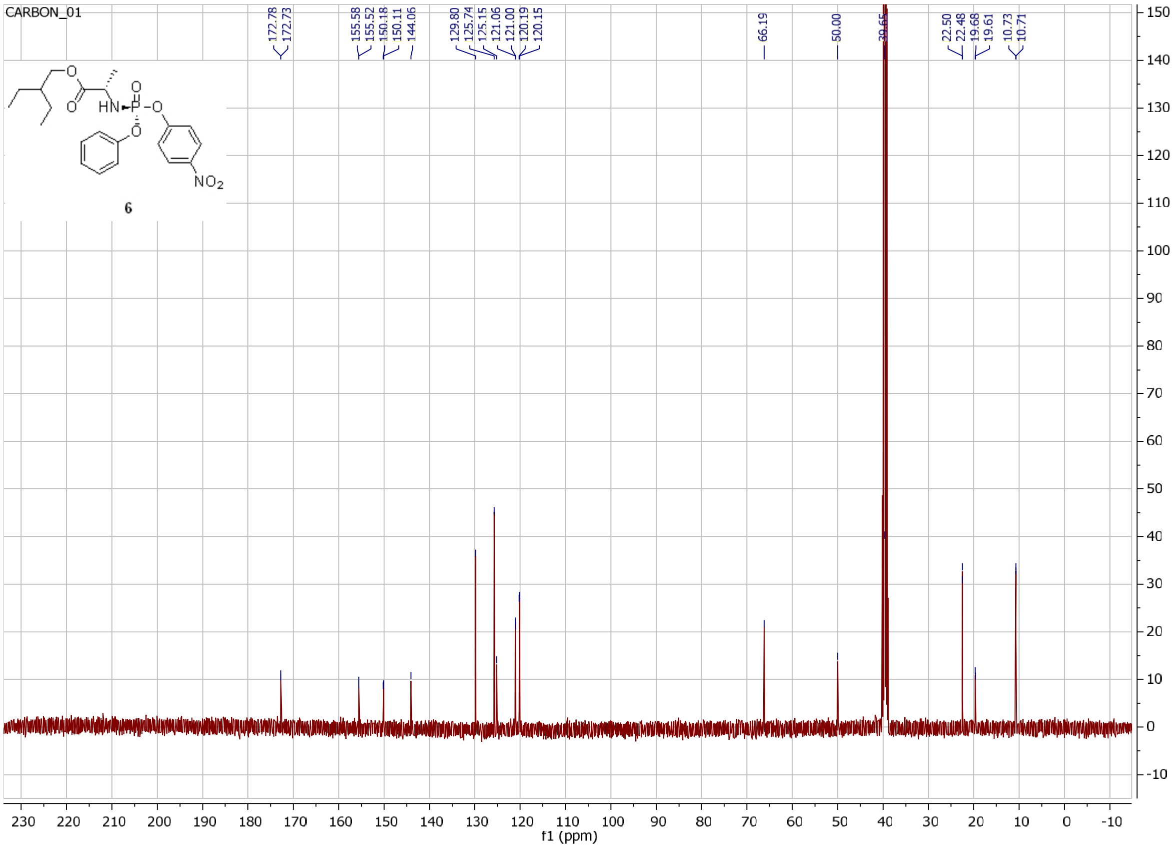
2.01
2.82
0.98
1.00
1.00
2.11
0.91
0.95
1.05
1.92
1.08
7.92
6.04
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)

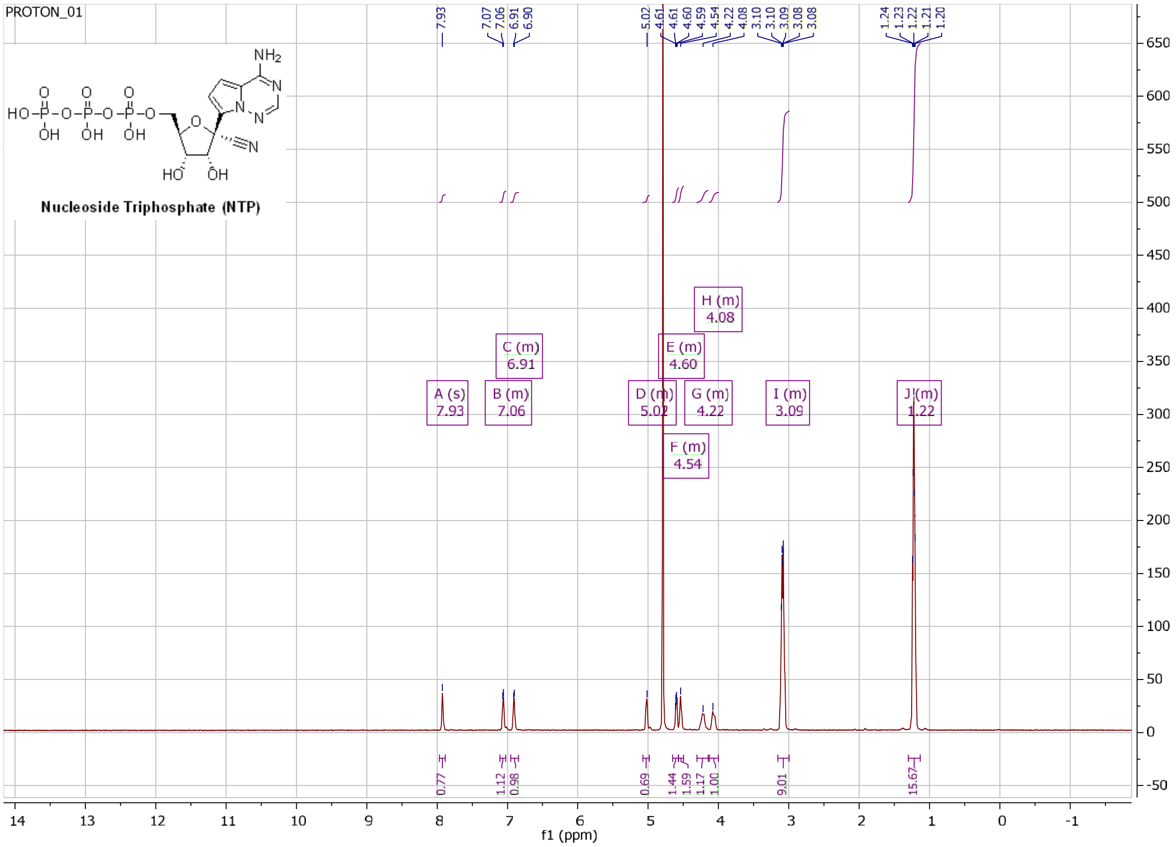

CARBON_01
174.98
174.92
157.18
152.14
152.07
148.27
130.68
126.04
125.51
121.33
121.28
117.90
117.58
112.29
102.60
84.31
84.22
81.26
75.63
71.63
68.10
67.17
67.12
51.46
41.65
24.19
20.56
20.50
11.33
11.28
NH2
N
N
N
O
O
O
HN
P
O
O
O
O
N
HO
OH
GS-5734
550
500
450
400
350
300
250
200
150
100
50
0
-50
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

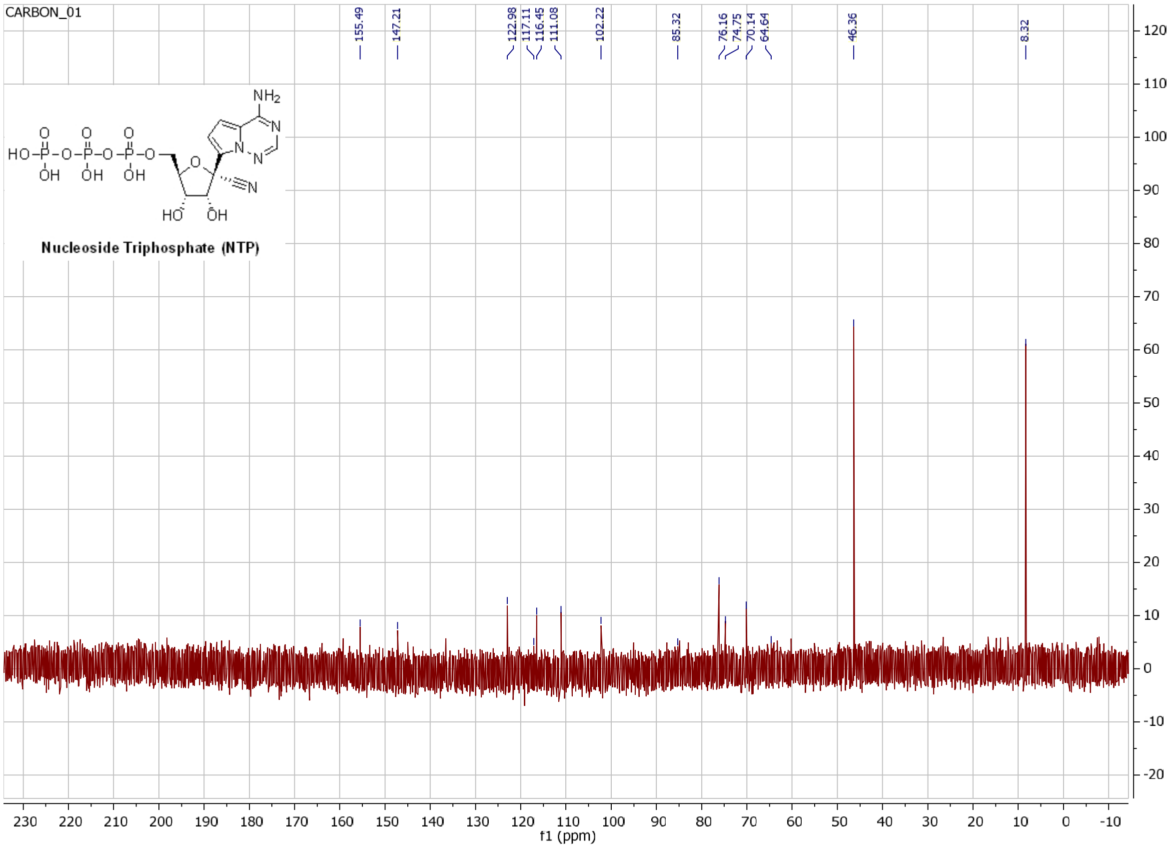

PROTON_01
HN
P
O
NO2
9
A (d) 8.22
B (dt) 7.37
C (m) 7.23
D (m) 4.15
E (m) 4.05
F (m) 3.91
G (m) 1.50
H (m) 1.37
I (t) 0.87
1.36
3.95
3.73
0.85
2.06
1.00
0.92
11.89
7.31
f1 (ppm)


CARBON_01
HN
P
O
NO2
9
173.27
173.24
173.18
173.16
155.68
155.63
155.62
155.57
150.39
150.33
150.27
129.97
129.95
129.95
125.70
125.67
125.60
120.94
120.92
120.89
120.87
120.23
120.19
120.18
120.14
67.89
67.87
50.63
50.61
40.33
40.32
23.26
23.24
21.17
21.13
21.08
11.03
11.01
f1 (ppm)


PROTON_01
8.32 8.31 8.31 8.29 8.29 8.28 7.52 7.52 7.51 7.51 7.50 7.50 7.49 7.49 7.48 7.42 7.41 7.40 7.39 7.38 7.24 7.23 7.22 7.22 7.21 7.20 6.71 6.69 6.68 6.66
4.04 4.03 4.02 4.01 4.00 4.00 3.99 3.98 3.97 3.95 3.93 3.91 1.43 1.42 1.40 1.39 1.37 1.28 1.27 1.27 1.25 1.25 1.24 1.24 1.24 1.23 1.23 1.22 1.21 0.81 0.79 0.79 0.77
HN
P
O
O
O
O
O
NO2
6
A (m) 8.30
B (m) 7.51
C (m) 7.40
D (m) 7.23
E (dd) 6.68
F (m) 4.01
G (d) 3.92
H (p) 1.40
I (m) 1.24
J (m) 0.79
1.93
1.94
1.96
2.85
1.00
0.86
1.94
1.01
7.16
8.98
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)
800 750 700 650 600 550 500 450 400 350 300 250 200 150 100 50 0 -50


CARBON_01
172.78 172.73
155.58 155.52 150.18 150.11 144.06
129.80 125.74 125.15 121.06 121.00 120.19 120.15
66.19
50.00
39.65
22.50 22.48 19.68 19.61 10.73 10.71
HN
P
O
O
O
O
O
NO2
6
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10


PROTON_01
Nucleoside Triphosphate (NTP)
NH2
HO
OH
CN
7.93
7.07
7.06
6.91
6.90
5.02
4.61
4.60
4.59
4.54
4.22
4.08
3.10
3.09
3.08
3.08
1.24
1.23
1.22
1.21
1.20
A (s) 7.93
B (m) 7.06
C (m) 6.91
D (m) 5.02
E (m) 4.60
F (m) 4.54
G (m) 4.22
H (m) 4.08
I (m) 3.09
J (m) 1.22
0.77
1.12
0.98
0.69
1.44
1.59
1.17
1.00
9.01
15.67
f1 (ppm)


CARBON_01
Nucleoside Triphosphate (NTP)
NH2
HO
OH
CN
155.49
147.21
122.98
117.11
116.45
111.08
102.22
85.32
76.16
74.75
70.14
64.64
46.36
8.32
f1 (ppm)

VI. References

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40. Mackman, R. L., Parrish, J. P., Ray, A. S. & Theodore, D. A. Methods and compounds for treating respiratory syncytial virus infections. *US Patent 2011045102*. Filed 22 Jul 2011.

41. Metobo, S. E. *et al.* Practical synthesis of 1′-substituted Tubercidin C-nucleoside analogues. *Tetrahedron Lett.* **53**, 484-486 (2012).